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Genotypic Association Between Dopamine Transporter Gene Polymorphisms and Schizophrenia

Antonio M. Persico* and Fabio Macciardi
Department of Neuropsychiatric Sciences, S. Raffaele Hospital, Milan, Italy

Dopamine transporter (DAT) gene variants do not appear to provide widespread contributions to the etiology of schizophrenia spectrum disorders, according to linkage studies [Persico et al., 1995: Am J Psychiatry 152:134–136]. They may, however, produce modifying effects, more readily detectable in specific subpopulations of schizophrenics through association analyses. We therefore compared polymorphic DAT gene variable number tandem repeat (VNTR) distributions in 84 controls and 147 patients, divided according to DSM-IIIR schizophrenia type criteria. No evidence of allelic association between DAT alleles and schizophrenia or any specific schizophrenia subtype was found. Interestingly, the DAT genotype distribution among schizophrenic patients did display a statistically significant departure from the genotype distribution found in controls. Such discrepancies may represent stigmata of assortative mating or may suggest a "modifying" contribution of homozygote DAT genotypes to pathogenetic processes underlying schizophrenia. *Am. J. Med. Genet. 74:53–57, 1997.*

KEY WORDS: dopamine; schizophrenia; dopamine transporter; association analysis; assortative mating

INTRODUCTION

The potential involvement of dopamine in the pathogenesis of schizophrenia has been intensely investigated for over two decades. The "dopamine hypothesis" of schizophrenia originated from initial evidence of dopamine receptor blockade exerted by antipsychotic drugs and of its correlations with efficacy on schizophrenic "positive" symptoms [Creese et al., 1976]. Subsequently, postmortem and *in vivo* dopamine receptor binding studies, assessments of dopaminergic turnover in schizophrenic patients, and clinical response to pharmacologic modulation of dopaminergic neurotransmission provided further evidence that dopamine system alterations may be present not only following chronic treatment with antipsychotic drugs, but also among untreated schizophrenics [Kahn and Davis, 1995]. Schizophrenia is presently viewed as a complex and heterogeneous disease, possibly stemming from neurodevelopmental derangements involving several neurotransmitters and neuromodulators [Waddington, 1993; Weinberger, 1995]. Within this neurodevelopmental framework, and despite the limitations of purely dopamine-based etiological hypotheses, hyperactive dopaminergic neurotransmission appears likely to characterize a significant number of individuals with schizophrenia and related disorders [Kahn and Davis, 1995].

The dopamine transporter (DAT) plays a pivotal role in dopaminergic neurotransmission, as it terminates dopaminergic activity in the synapse by taking released neurotransmitter back into the presynaptic terminal. Human DAT cDNAs have been cloned [Vandenbergh et al., 1992a], the DAT gene has been located on human chromosome 5p15.3 [Vandenbergh et al., 1992b], and a polymorphic 40-base variable number tandem repeat (VNTR) has been found in the genomic sequence encoding the mRNA 3′ untranslated region [Vandenbergh et al., 1992b]. In Caucasian- and in African-Americans, this VNTR copy number varies between 3–11, with >90% of the individuals displaying 9 or 10 copies [Vandenbergh et al., 1992b; Persico et al., 1993, 1996].

The existence of genetic contributions to the pathogenesis of a cluster of disorders falling within the "schizophrenia spectrum," including schizophrenia, schizophreniform, schizoaffective, and schizotypal disorders, and mood-incongruent psychotic depression, is supported by twin, adoption, and familial aggregation studies [Farmer et al., 1987; Kendler, 1988]. Genes producing proteins which play relevant roles in dopaminergic neurotransmission, such as the DAT locus, may

*Correspondence to: Antonio M. Persico, M.D., now at the Laboratory of Neuroscience, Libero Istituto Universitario Campus Bio-Medico, Via Longoni 83, I-00155 Rome, Italy.

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thus represent candidate genes for these disorders. Conceivably, specific DAT alleles might confer enhanced vulnerability to the development of schizophrenia spectrum disorders, or may significantly modify its onset and clinical course.

We previously found no evidence of linkage between DAT gene markers and schizophrenia spectrum disorder in 16 multiplex pedigrees [Persico et al., 1995]. These results do not support the existence of widespread and significant causal relationships between genetic mutations at the DAT locus and the disease phenotype. Nonetheless, linkage analysis suffers from relevant power limitations when applied to heterogeneous, possibly oligogenic disorders such as schizophrenia [Gershon et al., 1989]. Furthermore, it cannot detect potentially relevant "modifying" effects of DAT gene variants in these disorders. Association analyses, on the contrary, appear more powerful in detecting single-gene effects in heterogeneous polygenic diseases [Gershon et al., 1989]. Moreover, association approaches may reveal single-gene-induced "modifying" effects when genotypic distributions of candidate allelic variants are contrasted among groups of patients differing for specific clinical features [Persico et al., 1996] or even personality traits [Benjamin et al., 1996; Ebstein et al., 1996].

We report on the results of an association study performed on 84 population controls and 147 patients, divided into "paranoid," "undifferentiated," and "disorganized" according to DSM-IIIR schizophrenia type criteria.

MATERIALS AND METHODS

A total of 231 individuals of Italian descent was recruited, including 147 patients and 84 controls. Random sampling of schizophrenic patients was performed by enrolling individuals consecutively admitted for inpatient or outpatient treatment to the Department of Neuropsychiatric Sciences, S. Raffaele Hospital (Milan, Italy), diagnosed according to DSM-IIIR [American Psychiatric Association, 1987] criteria for schizophrenia and assessed using the SADS-L interview [Endicott and Spitzer, 1978]. Schizophrenia subtyping, according to DSM-IIIR criteria, was performed in 113 of the 147 patients. Predominance of male schizophrenics in our sample (see below), in contrast with typically balanced male/female ratios, may likely stem from collection of most patients during acute episodes of male-predominant early-onset schizophrenia requiring inpatient treatment. Schizophrenics were contrasted with 84 unassessed controls randomly collected from the general population among unrelated individuals referred to the laboratories of our Hospital by their general practitioner for blood tests related to nonpsychiatric medical illnesses. Both schizophrenics and controls are part of a larger sample of Italian individuals, whose combined DAT VNTR allelic distribution has been presented in a previous report, focused on interethnic stratification of DAT polymorphisms [Persico et al., 1996].

Blood samples were drawn from patients and controls, after obtaining informed consent; genomic DNA was extracted using the method of Lahiri and Nurnberger [1991]. Dopamine transporter gene markers were assessed using polymerase chain reaction (PCR), as previously described [Vandenbergh et al., 1992b]. Briefly, a 40-base pair (bp) variable number tandem repeat (VNTR) located in the 3′ untranslated region of the DAT cDNA was amplified from 40 ng of genomic DNA using 1.25 u of AmpliTaq DNA polymerase, through 35 cycles as follows: denaturing for 1 min at 93°C and annealing/extension for 1 min at 72°C, in buffer supplied by the manufacturer (Perkin-Elmer) and in the presence of primers 5′-TGTGGTG-TAGGGAACGGCCTGAG-3′ and 5′-CTTCCTGGAG-GTCACGGCTCAAGG-3′ at 5×10^{-7} M. Amplification products were electrophoresed using 5% polyacrylamide gels, and product sizes were determined by comparison with molecular weight standards (BRL).

Allelic and genotypic distributions in schizophrenics and controls were contrasted by chi-square tests, performed using the CONTING and CHIPROB programs [Ott, 1991].

RESULTS

Dopamine transporter VNTR genotypes in schizophrenics displayed significantly enhanced homozygote (genotypes 9/9 and 10/10), and reduced heterozygote (genotype 9/10) frequencies of the most common genotypes, when contrasted with controls (Table I). This

TABLE I. Dopamine Transporter VNTR Genotypic Frequencies in 147 Schizophrenics, Including 92 Males and 55 Females, and in 84 Population Controls*

DAT VNTR genotype	SCZ (N = 147)	Controls (N = 84)	Male SCZ (N = 92)	Female SCZ (N = 55)
5/5	1 (0.7%)			1 (1.8%)
6/6	1 (0.07%)		1 (1.1%)	
7/7	1 (0.7%)		1 (1.1%)	
9/9	25 (17.0%)	7 (8.3%)	19 (20.6%)	6 (10.9%)
9/10	48 (32.6%)	41 (48.8%)	29 (31.5%)	19 (34.6%)
10/10	69 (46.9%)	35 (41.7%)	40 (43.5%)	29 (52.7%)
9/11	1 (0.7%)	1 (1.2%)	1 (1.1%)	
10/11	1 (0.7%)		1 (1.1%)	

* SCZ, schizophrenics. SCZ vs. controls: $\chi^2 = 6.79$, 2 df, two-tailed $P = .034$. Male SCZ vs. controls: $\chi^2 = 7.79$, 2 df, two-tailed $P = .020$. Female SCZ vs. controls: $\chi^2 = 2.69$, 2 df, two-tailed $P = .261$. Male SCZ vs. female SCZ: $\chi^2 = 2.61$, 2 df, two-tailed $P = .272$.

TABLE II. Dopamine Transporter VNTR Allelic Frequencies in 147 Schizophrenics, and in 84 Population Controls*

DAT VNTR allele	SCZ (N = 294)	Controls (N = 168)
5	2 (.0068)	
6	2 (.0068)	
7	2 (.0068)	
9	99 (.3367)	56 (.3333)
10	187 (.6361)	111 (.6607)
11	2 (.0068)	1 (.0060)

* SCZ, schizophrenics. SCZ vs. controls: $\chi^2 = 2.58$, 2 df, two-tailed $P = .275$.

trend appeared in both male and female patients, although it reached statistical significance among male schizophrenics only, possibly due to differences in statistical power (Table I). Rare genotypes, which were excluded from chi-square analyses (see below), were also found almost exclusively among schizophrenic patients (Table I). At the same time, we found no evidence of allelic association with schizophrenia (Table II) or with disorganized, undifferentiated, or paranoid subtypes (Table III).

Enhanced homozygote frequencies did not appear to characterize any specific schizophrenia subtype in the overall sample (Table III). When genotypes were broken down by schizophrenia subtype and sex, however, male paranoid and female disorganized schizophrenics displayed trends toward enhanced homozygosity, with nonsignificant differences likely due to small sample sizes (Table IV).

DISCUSSION

The present results support the existence of a genotypic association between DAT VNTR polymorphisms and schizophrenia, in the absence of an allelic association. Schizophrenics present enhanced frequencies of the most common homozygote genotypes (i.e., 9/9 and 10/10), and a reduction in heterozygote 9/10 genotype frequency. This feature may be shared by patients of both sexes, although only male schizophrenics display a statistically significant genotypic association, possibly due to the smaller size of our female schizophrenic sample (Table I).

Our findings may potentially stem from at least two distinct processes. Enhanced homozygote and reduced heterozygote frequencies could be conservatively interpreted as the expected outcome of assortative mating [Cavalli-Sforza and Bodmer, 1971; Galbaud du Fort et al., 1994], which has indeed been shown to occur in schizophrenia [Maier et al., 1993]. The presence of rare genotypes almost exclusively limited to schizophrenic patients in our sample would lend support to this hypothesis. Caution is, however, required in interpreting genotypes produced by VNTR alleles shorter than 9 repeats. The frequency of these alleles in the general population is in fact so low, that their presence, always in homozygote form, reported in studies assessing altogether more than 2,000 chromosomes [Vandenbergh et al., 1992b; Li et al., 1994; Persico et al., 1996], may not reflect the actual existence of two copies of these rare alleles in the same individual, but may rather be produced through other mechanisms, such as preferential amplification of shorter tandem repeats. Rare alleles and genotypes have therefore not been included in our chi-square calculations on genotypic data.

Alternatively, homozygote DAT genotypes may contribute a "modifying" effect to the disease. Lod scores obtained for schizophrenia and schizophrenia spectrum disorder in linkage studies performed to date, under both dominant and recessive models, strongly argue against DAT allelic variants providing major gene contributions to the etiology of schizophrenia spectrum disorders [Byerley et al., 1993; Persico et al., 1995]. These linkage studies, however, display dramatic power limitations if genetic heterogeneity is taken into account [Persico et al., 1995]. Linkage analyses cannot exclude that allelic variants at the DAT locus may either represent a rare cause of schizophrenia or that they may have a "modifying" impact on specific features of the disease [Persico et al., 1995]. Both these tasks may be better undertaken using association analysis [Gershon et al., 1989]. Intriguing associations of this same DAT VNTR marker with cocaine-induced paranoia [Gelernter et al., 1994] and attention-deficit-hyperactivity disorder [Cook et al., 1995] have recently been reported. In an oligogenic disorder with significant environmental influences, such as schizophrenia, the effect of a single gene might conceivably account for clinical traits most readily detectable only in a specific subset of patients. Although we found no evidence of allelic or genotypic associations with specific DSM-IIIR schizophrenia subtypes, larger samples are necessary to replicate and confirm this finding. Possible differential contributions to disorganized and paranoid schizophrenia in female and male patients, respectively, may

TABLE III. Dopamine Transporter VNTR Genotypic and Allelic Frequencies in 113 Patients With Disorganized, Undifferentiated, or Paranoid Schizophrenia*

DAT VNTR Genotype	Disorganized (N = 40)	Undifferentiated (N = 43)	Paranoid (N = 30)	DAT VNTR allele	Disorganized (N = 80)	Undifferentiated (N = 86)	Paranoid (N = 60)
5/5	1 (2.5%)			5	2 (.0250)		
7/7		1 (2.3%)		7		2 (.0233)	
9/9	7 (17.5%)	6 (14.0%)	4 (13.3%)	9	25 (.3125)	28 (.3256)	17 (.2833)
9/10	11 (27.5%)	15 (34.9%)	9 (30.0%)	10	52 (.6500)	55 (.6395)	43 (.7167)
10/10	20 (50.0%)	20 (46.5%)	17 (56.7%)	11	1 (.0125)	1 (.0116)	
9/11		1 (2.3%)					
10/11	1 (2.5%)						

* Genotypes: $\chi^2 = 0.94$, 4 df, two-tailed $P = .919$. Alleles: $\chi^2 = 0.49$, 2 df, two-tailed $P = .782$.

TABLE IV. Dopamine Transporter VNTR Genotypic Frequencies in 69 Male and 44 Female Patients With Disorganized, Undifferentiated, or Paranoid Schizophrenia*

	Male Schizophrenics			Female Schizophrenics		
DAT VNTR Genotype	Disorganized (N = 23)	Undifferentiated (N = 29)	Paranoid (N = 17)	Disorganized (N = 17)	Undifferentiated (N = 14)	Paranoid (N = 13)
5/5				1 (5.9%)		
7/7		1 (3.4%)				
9/9	5 (21.7%)	4 (13.8%)	3 (17.6%)	2 (11.8%)	2 (14.3%)	1 (7.6%)
9/10	8 (34.8%)	10 (34.5%)	3 (17.6%)	3 (17.6%)	5 (35.7%)	6 (46.2%)
10/10	9 (39.1%)	13 (44.9%)	11 (64.8%)	11 (64.7%)	7 (50.0%)	6 (46.2%)
9/11		1 (3.4%)				
10/11	1 (4.4%)					

* Homozygotes (9/9) + (10/10) vs. heterozygotes (9/10): males, $\chi^2 = 2.12$, 2 df, two-tailed $P = .346$; females, $\chi^2 = 2.55$, 2 df, two-tailed $P = .280$.

also suggest potential sex-dependent expression of DAT-related contributions which deserve further investigation. Finally, DAT homozygote genotypes may provide clinically or biologically relevant contributions to the disease phenotype without necessarily exerting an impact on diagnostic subtyping of schizophrenia, as it stands in DSM-IIIR. Recent reports describing an association between dopamine D4 receptor gene polymorphisms and the personality trait of "novelty seeking" [Ebstein et al., 1996; Benjamin et al., 1996] spur interest in single genes predisposing to traits cutting across multiple polygenic disorders [Cloninger et al., 1996].

Case-control association studies can potentially be undermined by stratification. Distinct racial and ethnic groups display significant differences in DAT marker distributions [Persico et al., 1996]. Although patients and controls in this study belong to the same ethnic group, caution is needed until our results are independently replicated. In the only other association study published to date, Li et al., [1994] found no allelic or genotypic association between the same 40-bp VNTR employed in this study and schizophrenia. Whereas the lack of allelic association they reported confirms our findings, their sample of Chinese schizophrenics and controls is uninformative with regard to genotypic associations, because the Chinese population displays extremely high frequencies of the 10-repeat allele, and is thus much more homogeneous than the samples of Caucasian individuals assessed thus far [Persico et al., 1996]. Allelic distributions very similar to those found among the Chinese have also been recorded in a sample of Japanese controls [Sano et al., 1993]. Therefore, further association studies aimed at verifying the existence of DAT genotypic associations with schizophrenia or specific schizophrenia subtypes will have to be undertaken in more heterogeneous and informative Caucasian samples.

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